

Development of an active risk-based surveillance strategy for avian influenza in Cuba



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ABSTRACT

The authors designed a risk-based approach to the selection of poultry flocks to be sampled in order to further improve the sensitivity of avian influenza (AI) active surveillance programme in Cuba. The study focused on the western region of Cuba, which harbours nearly 70% of national poultry holdings and comprise several wetlands where migratory waterfowl settle (migratory waterfowl settlements – MWS). The model took into account the potential risk of commercial poultry farms in western Cuba contracting from migratory waterfowl of the orders *Anseriformes* and *Charadriiformes* through dispersion for pasturing of migratory birds around the MWS. We computed spatial risk index by geographical analysis with Python scripts in ESRI® ArcGIS 10 on data projected in the reference system NAD 1927–UTM17. Farms located closer to MWS had the highest values for the risk indicator p_j and in total 31 farms were chosen for targeted surveillance during the risk period. The authors proposed to start active surveillance in the study area 3 weeks after the onset of *Anseriformes* migration, with additional sampling repeated twice in the same selected poultry farms at 15 days interval (Comin et al., 2012; EFSA, 2008) to cover the whole migration season. In this way, the antibody detectability would be favoured in case of either a posterior AI introduction or enhancement of a previous seroprevalence under the sensitivity level. The model identified the areas with higher risk for AIV introduction from MW, aiming at selecting poultry premises for the application of risk-based surveillance. Given the infrequency of HPAI introduction into domestic poultry populations and the relative paucity of occurrences of LPAI epidemics, the evaluation of the effectiveness of this approach would require its application for several migration seasons to allow the collection of sufficient reliable data.

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1. Introduction

Until 1996, highly pathogenic avian influenza (HPAI) viruses belonging to serotypes H5 and H7 viruses were successfully eradicated or failed to persist in nature (Salomon and Webster, 2009). However, avian influenza (AI) has

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greatly enhanced its significance in the last years. It has been calculated that the impact of AI on the world-wide poultry industry from 1999 to 2004 (Capua and Alexander, 2004) involved more than 200 million animals. Today, it is unknown whether the ecology of these viruses has changed and whether highly pathogenic H5N1 viruses continue to be propagated in domestic or wild bird reservoirs.

Furthermore, some avian influenza virus (AIV) can infect humans with serious public health implications (WHO, 2013, 2014). Changes in agricultural practices, enhanced animal health surveillance, and/or virus evolution may have contributed to the apparent increase in animal influenza outbreaks reported in recent times; that turns AI into an increasing concern for veterinarians worldwide (Ducatez et al., 2008).

The surveillance and control of AI have historically focused on the detection and eradication of infections due to HPAI viruses in poultry populations. However, reports of low pathogenicity avian influenza (LPAI) viruses in poultry are recurrent, with outbreaks annually affecting several countries (WAHIS, 2014). Most of AI infections in poultry are caused by LPAI virus strains, which may belong to any serotype, including H5 and H7. These H5 or H7 LPAI viruses may circulate causing unnoticeable clinical signs, unless they mutate into HPAI viruses (Alexander and Brown, 2009). Therefore the recurrence of LPAI virus circulation is a constant risk to poultry industries throughout the world.

Various approaches have been applied for the diagnosis of AI (OIE, 2008), including techniques for the detection of the virus, its genome, antigens or antibodies. However, the antibodies to AIV, as evidence of infection, often persist for the entire production life of the infected poultry (Fouchier et al., 2003), allowing a high opportunity for long-term diagnosis. The detection of a significant increase of antibody titre allows an opportunity for early warning. Consequently, antibody detection to LPAI viruses is compulsory for several countries, e.g. in the EU countries (European Commission, 2007, 2009).

The control and eradication of AI are based on passive and active surveillance, disease notification, prevention of possible contacts, biosecurity measures, and movement restriction of live birds, poultry products, by-products and potentially infective material, and depopulation of infected farms (OIE, 2013). However, when timely disease detection fails, the stamping out as a key control measure could become ineffective because the virus could be already seriously disseminated into vast poultry populations. In such cases, the economical consequences of the outbreak could be devastating.

Wild waterfowl (particularly ducks, geese, swans, gulls and shorebirds) are considered the original source of all AIV known subtypes (Munster et al., 2007). Hence the active surveillance, aimed at early detection of the disease, in several countries or regions, has included the sampling of wild bird (Burns et al., 2012; Iglesias et al., 2010). However, wild birds are not well suited for active surveillance for a number of reasons. AI virus in wild waterfowl shows a seasonal prevalence, a very variable pattern, which can vary over time and between locations within a species (Olsen et al., 2006; Figuerola et al., 2007;

Hill et al., 2012). It is, therefore, difficult to make an initial assessment of the most important species to target on the basis of virus detection alone, which demands high sampling intensity for detecting viruses.

Furthermore, sampling of wild birds is a labour-intensive, costly, and time-consuming task that has not been exempted from discussion at the decision-making level in the European Union and other regions affected by the disease (Martinez et al., 2011). The detection of viruses in migrating birds does not necessarily mean that these viruses have been, or will be, successfully introduced into a new geographic area (Martin et al., 2009) and, consequently, resident waterfowl could be a best target to assess the establishment of AI in a geographical area. However, sampling and testing of wild birds is not required by the Terrestrial Animal Health Code to declare a country, zone or compartment free from AI (OIE, 2013).

The design of surveillance programmes has to be carefully planned, taking into consideration the local epidemiological and ecological conditions, the areas where migrating waterfowl transit and settle (Miller et al., 2009; OIE, 2013; U.S., 2007, 2008), and social and economic conditions (Alfonso et al., 2008; Fiebig, 2011; Martin et al., 2011; Stärk et al., 2006).

The geographical location of Cuba makes this island an important site along bird migration routes for resting or wintering (Blanco, 2006) (Fig. 1).

The Cuban poultry population susceptible to AIV comprises 14 million of poultry, predominantly reared for egg production, which constitute an important source of proteins of animal origin for residents.

People living in rural areas of Cuba raise poultry, mainly for own consumption rather than for commercial purposes. Details of the structure of Cuban poultry farming are reported in the Supplementary Document 1. In summary, 88% of Cuban poultry belong to commercial farms while the rest of the poultry rearing has a low average density (around 17.4 birds/km²).

Cuban AI surveillance programme focuses on determining either the evidence or the presence of infections by subtypes H5 and H7, as those of main concern for poultry due to its ability to become highly pathogenic after transmission to alternative hosts (Martin et al., 2009). This approach is in agreement with the chapter on AI of the Terrestrial Animal Health Code (OIE, 2013). Currently, the AI surveillance in Cuba is based on a passive and an active component.

The passive surveillance is usually the most effective for early detection of exotic diseases with severe clinical forms, such as the HPAI. It is, however, less effective in detecting the LPAI strains and it requires laboratory confirmation and typing of the virus strain responsible for the outbreak. The active component is based on serology by inhibition of hemagglutination assay (IHA), which is designed to be able to detect at least a prevalence of 5% AI infected holdings, with 30% infected animals within an infected holding (IMV, 2006). These values of target prevalence can lead to missing the presence of infection or delay in its detection, depending on the dynamics of the infection in the population (Gonzales et al., 2010). However, the sensitivity of active surveillance can

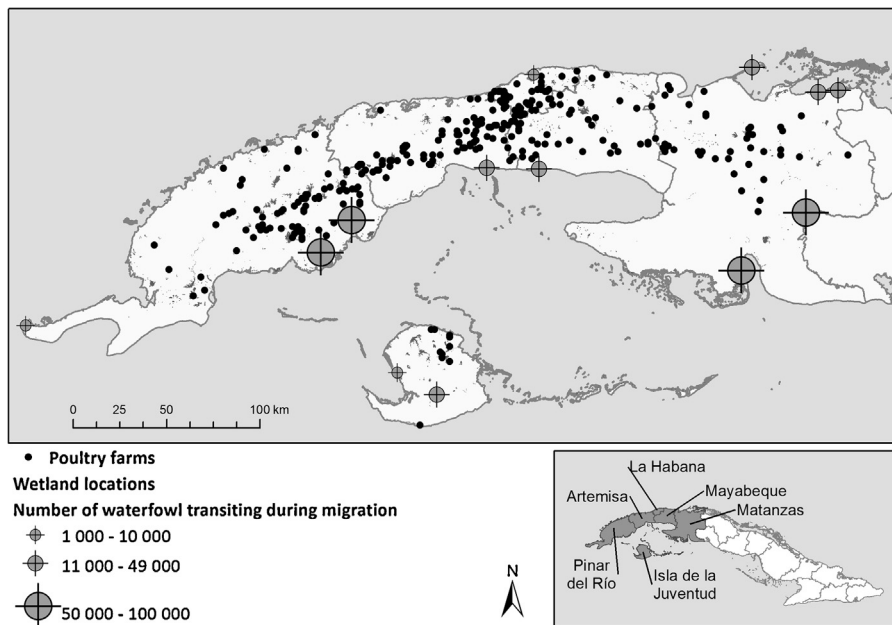


Fig. 1. Geographical distribution of poultry farms (black dots) and geographical location of wetlands (centroid of the area) in the western part of Cuba. The size of the points locating the wetlands is proportional to the number of waterfowl transiting during migration.

be improved using a risk-based surveillance (Cameron, 2012; Salman, 2003; Thrusfield, 2005). This, in the case of AI can be implemented through a risk-based selection of poultry farms to be surveyed, and through the concentration of surveillance activities during the period at risk for AI introduction, i.e. during waterfowl migration.

Aim of this paper is to describe the design of the risk-based surveillance and the criteria used to choose which poultry flocks have to be surveyed.

2. Materials and methods

2.1. Study area

The study area is the western region of Cuba. This region includes five provinces named Pinar del Río, Artemisa, Mayabeque, La Habana, Matanzas, and Isla de la Juventud (Fig. 1). It covers nearly 29% of the country surface and harbours approximately 70% of the poultry commercial holdings of Cuba. In this area, the consequences of a possible AI introduction would be more devastating than elsewhere in Cuba, as mentioned by Rutten et al. (2012) for zones of high poultry concentration.

This geographical area contains several wetland areas (Fig. 1), including the largest one of the Caribbean region, Ciénaga de Lanier y Sur de la Isla de la Juventud (RAMSAR <http://ramsar.wetlands.org/>), which harbours several waterfowl species implicated as AIV reservoir (Acosta and Mugica, 2006; Blanco, 2006; Munster et al., 2007; Olsen et al., 2006).

2.2. Source of data

2.2.1. Poultry data

“Location” (geographical coordinates) and “census” data from each of the 300 poultry farms registered in the western region of Cuba were obtained from the National Poultry Register and National Veterinary Service. Backyard poultry are not included. For each farm, information on biosecurity were also collected, focusing on inadequate anti-bird netting or drinking water supply accessible by wild birds.

The 300 commercial poultry farms in the census used in this study (dark and light dots in Fig. 2 and Fig. s3 in supplementary material) are located in the central part of the regions of Pinar del Río, Artemisa, Mayabeque, Matanzas, and widely distributed in La Habana province facing the northern coast. In the island ‘Isla de la Juventud’, poultry farms are located in the northern part, being the southern part of a national park.

2.2.2. Migratory waterfowl data

Data on the abundance of wild birds belonging to the orders of *Anseriformes* and *Charadriiformes* in the western part of Cuba, have been described in previous ornithological studies (Acosta and Mugica, 2006; Blanco, 2006). Data used in this study have been obtained from these previous studies and summarised in Table 1.

2.3. Statistical analysis

2.3.1. Descriptive statistics

For each wetland, the mean of the number of migratory waterfowl transiting during migration was associated to

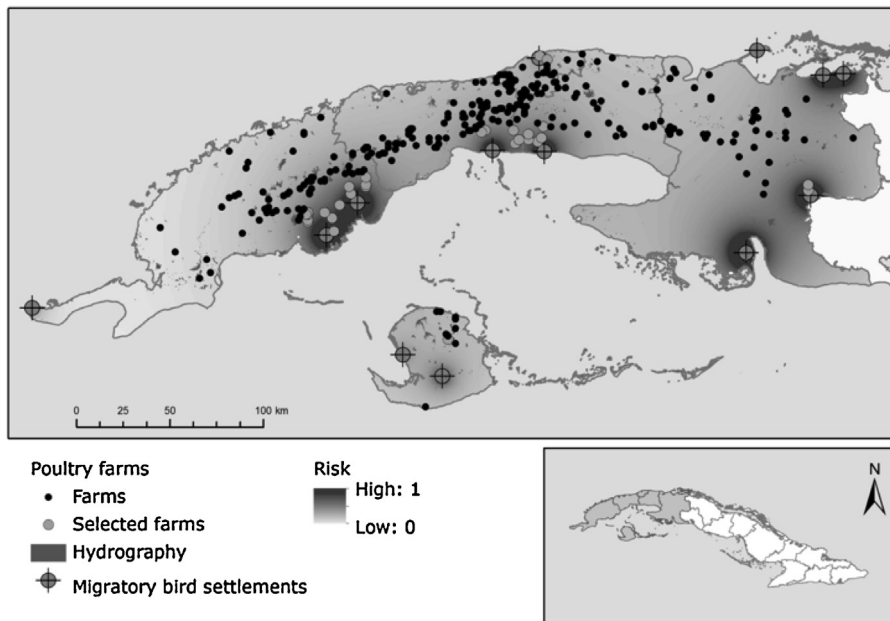


Fig. 2. Risk map of introduction of avian influenza through migratory waterfowls (settlements with cross hairs dots) in the Western region of Cuba and selected farms (light dots) for active surveillance programme.

the coordinates of the centroid of the wetland. Data from [Guberti \(2006\)](#) on the frequency distribution of displacement distances have been used to model the displacement of migratory waterfowl around the wetlands for pasturing. An inverse distance function was regressed on [Guberti \(2006\)](#) raw data and then rescaled to 0–10 km to adapt the European data to Cuban situation. Ten kilometres have been considered the maximum range of daily movement of wild birds for pasturing around the arrival settlement in the Cuban ecological system ([Blanco, 2006](#)). The probability of pasturing at larger distances was considered very low or negligible.

2.3.2. Spatial model

For each migratory waterfowl settlement, the daily pasturing dispersal in function of the distance is:

$$f(d) = \frac{0.34063782}{d}$$

where: d is the distance in km, and with the constrain: $\forall d: 0 < d < 1 f(d) = f(1)$

0.34063782 is a conversion factor to rescale the inverse distance function regressed on [Guberti \(2006\)](#) raw data to the 0–10 km range relevant for the Cuban environment.

Table 1

Migratory waterfowl settlements in the Western part of Cuba: location, abundance of wild birds from genera (*Charadriiformes*, [Blanco, 2006](#), and *Anseriformes*, [Acosta and Mugica, 2006](#)) associated to avian influenza transmission.

Progressive number	Province	Name of the setting	Setting position		Number of birds passing during migration	Criteria of abundance	Number of species
			Longitude W	Latitude N			
1	Matanzas	Amarillas	–80,54,14	22,28,53	50,000–100,000	High	29
2		Las Salinas, C. Zapata	–81,14,19	22,12,10	50,000–100,000	High	74
3		Península de Hicacos	–81,10,59	23,10,59	11,000–49,000	Medium	67
4		Salinas de Bidos	–80,44,00	23,04,18	11,000–49,000	Medium	49
5		Laguna del Concunil	–80,50,17	23,03,48	11,000–49,000	Medium	24
6	Mayabeque	Batabanó	–82,17,32	22,41,16	11,000–49,000	Medium	14
7	Artemisa	Guanimar	–82,33,45	22,41,27	11,000–49,000	Medium	22
8	La Habana	Tricornia	–82,19,25	23,08,28	1000–10,000	Low	33
9	Pinar del Río	Alonso Rojas	–83,25,16	22,16,18	50,000–100,000	High	52
10		Sur los Palacios	–83,15,46	22,25,47	50,000–100,000	High	52
11		Guanahacabibes	–84,56,18	21,53,24	1000–10,000	Low	55
12	I. Juventud	Ciénaga de Lanier	–82,48,36	21,35,45	11,000–49,000	Medium	29
13		Los Indios	–83,00,56	21,41,54	1000–10,000	Low	19

A raster of 1 km × 1 km spatial resolution has been created in which for each pixel, the risk posed by waterfowl in neighbouring wetlands p_j is proportional to the value:

$$P_j \propto \sum_{i=1}^{12} f(d_{j,i}) \times b_i \times \alpha_i$$

where: i = serial number of wetlands $i = 1, \dots, 12$; $d_{j,i}$ = Euclidean distance of pixel j from the wetland i ; b_i = mean number of wild waterfowl passing through the wetland i during the migration period (Table 1); α_i = multiplication factor for the effective pasturing area available to migratory birds, calculated as the ratio between the area of dry land in 10 km buffer around the waterfowl settlement and the area of a circle of 10 km radius.

The factor α_i takes into account the location of the migratory waterfowl settlements, often very close to the Cuban coastline. In this case, the bird population will spread over the dry land only, so the density has been rescaled. The migratory waterfowl (MW) settlements reported in Western part of Cuba are 13 (Table 1) but that in Peninsula de Hicacos (Matanzas province) was excluded from the analysis as it is in a very thin and long peninsula and is in a touristic area (Acosta and Mugica, 2006). The geographical analysis has been performed using Python scripts in ESRI® ArcGIS 10, on data projected in the reference system NAD 1927–UTM 17.

2.4. Selection criteria

The selection of farms to be sampled throughout active surveillance, during seasonal waterfowl migration, was based on the index resulting from statistical analysis, which considered proximity to the migratory waterfowl settlement and density of MW. The values of the index p_j in each pixel have been normalised between 0 and 1. The risk for each farm, based on its location, was considered proportional to the value of p_j in the pixel corresponding to the centroid of the poultry farm. Any farm with an index (p_j) higher than 0.25 has been selected to be surveyed.

The aim of this risk-based surveillance system was to improve the sensitivity of surveillance for incursions of avian influenza (AI) in Cuba. In order to increase the chances and the timeliness of detection of possible incursions, the choice of farms to be included in the system was based on their vulnerability. Therefore, the presence of two other vulnerability factors, besides the exposition to migratory waterfowl was considered: the presence of (1) inadequate anti-bird netting or (2) drinking water supply from sources exposed to the contact with wild birds. The presence of either of these two biosecurity flaws was considered an ancillary criterion since in the absence of infection in MW, they would have been unable to trigger an outbreak. Their intended use was to provide further information for the selection of farms to survey in case of widespread ties in the scoring determined by the proximity with wetlands (index p_j).

3. Results

3.1. Risk distribution pattern

The geographical distribution of the index p_j (Fig. 2 and Fig. s3 in supplementary material) shows that the highest risk of introduction of AI is concentrated predominantly along the southern coastline of the study area, where most of wetlands with largest values of MW census are located.

The farms located closer to MW settlements had the highest values for the risk indicator p_j . Those with a p_j value greater than 0.25 were chosen for targeted surveillance during the risk period. There were no ties in the distribution of p_j values; therefore the selection of farms for risk-based surveillance did not make use of the two ancillary criteria on biosecurity.

3.2. Active surveillance strategy

According to selection criteria, 28 poultry farms were chosen. These did not include any in Havana province nor in the Isla de la Juventud municipality because the value of the risk indicator was below the threshold criterion in these locations (<0.25). Since these areas are densely populated, a possible introduction of AI could have significant consequences for public health, therefore 3 more farms were added from these provinces to the selected poultry flocks. They were chosen on the basis of their risk value, close or immediately below 0.25. Thus, the target poultry farms to be sampled were 31 (identified in Fig. 2 and Fig. s3 in supplementary material with light dots).

4. Discussion

The risk-based surveillance is characterised by the selection for sampling of populations or subpopulations where the disease presence is more likely and where prior risk factors exist (Cameron, 2012; Salman, 2003; Thrusfield, 2005). This may increase the probability of diseases detection. This type of monitoring is also used to document the absence of a disease in a population with a high degree of confidence (Salman, 2003).

Several risk factors are considered relevant as risk of AI transmission to poultry (Iglesias et al., 2010). In areas where the AI is exotic, the most relevant risk factors to be considered are those relating to the introduction of the infection. The main sources of infection for a free poultry population are migratory birds (especially waterfowl) and the trade of animals. In the Cuban situation, the main potential source of infection is migratory birds and the proximity to waterfowl settlements may enhance the probability of contact reservoir–poultry. Also important are breaches in biosecurity (FAO, 2008), which favour the direct or indirect contact between migratory birds and domestic poultry.

Concerning the other possible route of introduction of AI, due to the water isolated condition of the country, there is a moderate to low level of poultry and poultry product importation, always under strict veterinary control, so migratory waterfowl remain the most probable source of AIV introduction.

On the other hand, it is demonstrated that H5 and H7 avian influenza viruses, including highly pathogenic strains, have the ability to persist in water with wide variety of temperature and salinity for extended periods of time (Brown et al., 2007), hence it also emphasised the importance of the poultry water intake from natural lakes or ponds as a factor that must enhance the probability of AIV introduction in poultry farms.

Considering that the development of HI antibodies in detectable amounts requires at least 7 days post infection (EFSA, 2008), and the likely delay in AI transmission from MW to poultry (Ducatez et al., 2008), the authors proposed to start active surveillance in the study area 3 weeks after the onset of *Anseriformes* migration. Additionally, sampling must be repeated twice in the same selected poultry farms at 15 days interval (Comin et al., 2012; EFSA, 2008) to cover the whole migration season. In this way, the antibody detectability would be favoured in case of either a posterior AI introduction or enhancement of a previous seroprevalence under the sensitivity level.

The current surveillance programme for AI in Cuba consists of a passive component based on direct virus detection (IMV, 2006) in clinical suspects, mainly by real-time reverse transcription-PCR. The active component of this programme is based on a yearly random sampling of the 25% of poultry farm, with a within flock target prevalence of 30% (Ferrer et al., 2013). However, depending on the dynamics of infection in the affected holding (Gonzales et al., 2010) and the subsequent transmission between farms, the early detection may not be very efficient. It is expected that complementing the existing active surveillance with the targeted sampling of poultry holdings at high risk of contact with waterfowl, during the migration periods, would increase the chances for detecting AI introduction.

In Cuba as in several American countries, rice fields are important for water birds (Acosta et al., 2010; Mugica et al., 2006). However, rice cultivation shows seasonality and the places could vary according to land uses; therefore further studies are required to establish its importance relative to wetland for the transit and resting of MW during migration seasons. Anyway, considering that rice cultivation in the study region is mainly concentrated in the south (Martin et al., 2009) it can be hypothesised that the inclusion of rice padding in the model would lead to minor modifications of the risk map for AI introduction.

This work also considered commercial poultry farms for sampling as sentinels instead of backyard poultry during targeted surveillance. The role of backyard poultry in the spread of the AI is disputed according to several consideration of population size and breeds (Goutard et al., 2012). Backyard poultry are important in Asian countries in which they represent over 80% of the poultry population (Lee et al., 2008; Sedyaningsih et al., 2007). In Cuba, backyard poultry represent only 12% of the total poultry population. In densely populated poultry area of Northern Italy, backyard free-range farming is at high risk for introduction of avian influenza (Terregino et al., 2007), nonetheless, Bavinck et al. (2009), demonstrated that the probability of infection was much smaller for backyard flocks than for commercial farms in the 2003 Dutch epidemic by H7N7. For

this reason and for the low proportion of backyard poultry in respect to the commercial farming, the risk-based surveillance in Cuba considered only commercial poultry industry.

5. Conclusions

The model identified the areas with higher risk for AIV introduction from MW, for the selection of poultry premises where to apply the risk-based surveillance. Since the incursions of HPAI do not occur with a regular frequency and several years may elapse before the introduction of the virus, the evaluation of the effectiveness of this approach would require its application for several migration seasons before sufficient reliable data are collected.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.prevetmed.2014.05.012>.

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